



**AN INVESTIGATION OF THE (4;11)(q21;p15)
TRANSLOCATION IN ACUTE LYMPHOCYTIC
LEUKAEMIA**

by

Damian J. Hussey, B.Sc. (Hons)

Thesis submitted for the degree of
Doctor of Philosophy
to
The University of Adelaide
Department of Medicine
(The Queen Elizabeth Hospital)

June 2000

This copy is printed on archival paper

Table of Contents

Table of Contents	iv
List of Tables	ix
List of Figures.....	ix
Abstract.....	x
Declaration	xii
Acknowledgments	xiii
Publications	xv
Selected conference presentations arising from this thesis.....	xvi
Abbreviations	xviii
Chapter 1	
Introduction.....	2
1.1 Haematopoiesis	2
1.1.1 Classification of the leukaemias.....	3
1.1.1.1 Morphological and cytochemical criteria for leukaemia classification	4
1.1.1.2 Immunophenotyping	5
1.1.1.3 Cytogenetics.....	6
1.1.1.4 Proposed revised classification of the haematological malignancies	6
1.1.2 Incidence of leukaemic subtypes.....	7
1.2 Chromosome translocations and leukaemia	9
1.2.1 Chromosome translocations alter normal patterns of gene expression	9
1.2.1.1 Deregulating the expression of a proto-oncogene	10
1.2.1.2 Production of an in-frame fusion gene.....	11
1.2.2 Common targets of chromosome translocations	13
1.2.2.1 Disruption of transcription factors	13
1.2.2.2 Disruption of protein kinases	14
1.2.3 Rearrangements associated with T-cell ALL	15
1.3 Identifying genes at chromosome translocation breakpoints	17
1.3.1 Positional cloning approach.....	17
1.3.2 Candidate gene approach	18
1.4 Aims.....	19
1.4.1 Identification of a novel T-cell ALL translocation, t(4;11)(q21;p14-15).....	20
1.4.2 Preliminary molecular analysis of t(4;11)(q21;p14-15).....	22
1.4.3 Narrowing the chromosome 11 breakpoint region.....	23

Chapter 2

Materials and Methods	25
2.1 Materials	25
2.1.1 Buffers and Solutions:.....	25
2.1.2 Media	27
2.2 Methods.....	27
2.2.1 Basic nucleic acid isolation and manipulation procedures	27
2.2.1.1 Isolation of Lymphocytes/ mononuclear cells from peripheral blood.....	27
2.2.1.2 Thawing of frozen samples	28
2.2.1.3 DNA isolation from cell suspensions	28
2.2.1.4 RNA isolation procedure.....	29
2.2.1.5 Plasmid DNA isolation	29
2.2.1.6 DNA ligation.....	30
2.2.1.7 Preparation of competent <i>E. coli</i> and transformation with plasmid DNA.....	30
2.2.1.8 Sequencing	31
2.2.2 Cell culture.....	31
2.2.2.1 Thawing of cells for cell culture	31
2.2.2.2 Subculturing of NIH-3T3 mouse fibroblast cells	32
2.2.2.3 Transfection of NIH3T3 cells with plasmid DNA	32
2.2.3 Screening the λ gt11 bacteriophage library.....	33
2.2.4 Southern Analysis	34
2.2.5 Northern Analysis	36
2.2.6 Polymerase chain reaction (PCR) protocols.....	36
2.2.6.1 Standard PCR conditions	36
2.2.6.2 PCR conditions using Expand™ Long template and Expand™ Hi-Fi PCR System.....	37
2.2.6.3 Sequence of oligonucleotides for hybridisation, PCR and sequencing applications	37
2.2.6.4 Restriction endonuclease digestion of PCR products.....	40
2.2.6.5 PCR product purification	40
2.2.6.6 Reverse transcription (RT)- PCR conditions using MMLV.....	41
2.2.6.7 Reverse transcription (RT)- PCR conditions using Superscript II	41
2.2.6.8 DNase I treatment of RNA.....	42
2.2.7 Procedure for 3' RACE.....	42

Chapter 3

Investigation of the candidate gene, <i>ZNF195</i>.....	45
3.1 Confirmation of the 11p15.5 breakpoint region.....	47
3.2 Subcloning of zinc finger containing sequences from cosmid Z104.....	48
3.3 Are the zinc finger coding sequences present within Z104 transcribed?	51
3.3.1 Northern analysis of adult and foetal tissues using the 336 bp <i>Eco</i> RI fragment as a probe	53
3.3.2 Assessing the specificity of the 336 bp <i>Eco</i> RI fragment for Z104.....	53
3.3.3 Elevated expression of a 4.3 kb transcript in the t(4;11) patient	57
3.4 Isolation of lambda phage clones from a HUT-78 cDNA library	57
3.5 Assembly of restriction fragment and phage sequence into a contiguous sequence	58
3.6 Description of the <i>ZNF195</i> gene present within Z104	59
3.6.1 Determination of the intron/exon boundaries of <i>ZNF195</i>	61
3.6.2 Positioning of <i>ZNF195</i> within the Z104 cosmid	63
3.7 Alternative splicing of exons 4a and 4b.....	64
3.8 Revised Northern analysis with a probe from the 3'UTR of <i>ZNF195</i>	66
3.9 Discovery of a deletion/insertion polymorphism in the <i>ZNF195</i> 3'UTR.....	69
3.10 Assessing the imprinting status of <i>ZNF195</i>	71
3.11 Discussion.....	75
3.11.1 Northern analysis of <i>ZNF195</i> expression.....	75
3.11.2 Alternative splicing of <i>ZNF195</i>	76
3.11.3 What are the functions of <i>ZNF195</i> ?.....	76
3.11.4 Genomic position of <i>ZNF195</i>	77
3.11.5 Is <i>ZNF195</i> the 11p15.5 breakpoint gene in t(4;11)?.....	78

Chapter 4

Identification of the t(4;11)(q21;p15) breakpoint genes	81
4.1 Investigating <i>NUP98</i> as a candidate breakpoint gene in t(4;11)(q21;p15).....	82
4.1.1 Northern analysis	82
4.1.2 Southern analysis	84
4.1.3 Testing der(4) and der(11) for retention of <i>NUP98</i> sequences flanking the t(7;11)(p15;p15) breakpoint.	85
4.1.3.1 Primer pairs 5' of the published <i>NUP98</i> breakpoint	86
4.1.3.2 Primer pairs 3' of the published <i>NUP98</i> breakpoint	87
4.2 Identification of the chromosome 4 breakpoint gene	89
4.2.1 3' RACE to determine the sequence fused to <i>NUP98</i>	91
4.2.1.1 Characterisation of smaller RACE products	96

4.3 RT-PCR.....	98
4.4 Northern analysis	100
4.5 Discussion	103
4.5.1 Common features of t(4;11) ALL patients.....	105
4.5.2 t(4;11)(q21;p15) fuses the <i>NUP98</i> and <i>RAP1GDS1</i> genes	105
4.5.3 <i>NUP98</i> breakpoints	106
4.5.4 Fusion partners of <i>NUP98</i>	107
4.5.5 Retention of the FG repeat region in fusions involving nucleoporins.....	109
4.5.6 The role of smgGDS	109
Chapter 5	
Cellular localisation of nrg and its components	114
5.1 Creating constructs for studying the cellular location of nrg	115
5.1.1 Discovery of a novel exon in the predominant isoform of <i>RAP1GDS1</i>	117
in PBMNC	117
5.1.1.1 Other sequence variations in <i>RAP1GDS1</i>	121
5.1.2 Three fragment ligation for the creation of the gfp-nrg expression construct.....	122
5.1.3 <i>NUP98</i> cloning.....	124
5.2 Cellular localisation of gfp tagged nup98, nup98t, smgGDS and nrg.....	130
5.3 Discussion.....	133
5.3.1 The predominant isoform of smgGDS in PBMNC contains 12 armadillo repeats	133
5.3.2 Localisation of gfp-nup98.....	134
5.3.3 Localisation of gfp-nup98t.....	136
5.3.3.1 Possible disruption of crm1 mediated nucleocytoplasmic transport	137
5.3.3.2 Possible disruption of rae1 mediated nucleocytoplasmic transport.....	139
5.3.4 Localisation of gfp-smgGDS	140
5.3.5 Localisation of gfp-nrg.....	141
5.3.5.1 Nrg in the nucleus	142
5.3.5.2 The role of SMAP.....	146
5.3.5.3 Nrg in the cytoplasm.....	147
5.4 Conclusion	148

Chapter 6	
Conclusions and future studies	150
6.1 A common theme for nup98 fusions	151
6.1.1 Cbp and p300 are key regulators of haematopoietic differentiation	152
6.1.1.1 Cbp/p300 interacts with aml1	152
6.1.1.2 Haploinsufficiency of cbp causes haematopoietic defects	153
6.2 What is the role of the fusion partners of nup98?	153
6.2.1 Why is nrg specific to T-cell ALL?	155
6.3 Future studies	156
6.3.1 Identifying proteins that interact with nrg and pathways affected by nrg	156
6.3.1.1 A common pathway for T-cell ALL.....	157
6.3.2 Does nrg effectively cause increased expression/activity of smgGDS?.....	158
6.3.3 Further studies on nrg localisation	159
6.3.4 Assessing the transforming properties of nrg.....	159
6.3.4.1 <i>in vitro</i> transformation studies.....	160
6.3.4.2 <i>in vivo</i> transformation studies	161
References.....	164
Appendix Journal Papers.....	191

Abstract

This thesis describes the results of an investigation to determine the molecular basis of an uncharacterised (4;11)(q21;p15) translocation in a patient with T-cell acute lymphocytic leukaemia (ALL). The chromosome 11 breakpoint had been narrowed to the region between the markers *D11S860* and *D11S470*.

A new zinc finger gene near the breakpoint region was cloned and characterised. This gene, subsequently named *ZNF195*, encodes an N terminal KRAB domain and 14 tandemly repeated Krüppel type zinc finger motifs. *ZNF195* was subsequently localised distal to *D11S470* and therefore distal to the chromosome 11 breakpoint in the (4;11)(q21;p15) translocation. This excluded disruption of *ZNF195* by the translocation.

Subsequently the nucleoporin 98 gene (*NUP98*) was identified at an acute myeloid leukaemia translocation. *NUP98* maps distal to *D11S470* and therefore within the breakpoint region. Analysis of somatic cell hybrids segregating the t(4;11) translocation chromosomes showed that the chromosome 11 breakpoint occurred within *NUP98*. The fusion partner of *NUP98* was identified as the *RAP1GDS1* gene using 3' RACE. In the *NUP98-RAP1GDS1* fusion transcript (abbreviated *NRG*), the 5' end of the *NUP98* gene is joined in frame to the coding region of the *RAP1GDS1* gene. This joins the phenylalanine-glycine (FG) repeat rich region of *nup98* to *smgGDS* (the most common name for the protein encoded by *RAP1GDS1*) which largely consists of tandem armadillo repeats. *NRG* fusion transcripts were detected in the leukaemic cells of two other adult T-cell ALL patients with a t(4;11)(q21;p15) translocation. This is the first report of a *NUP98* translocation in lymphocytic leukaemia and the first time that *RAP1GDS1* has been implicated in any human malignancy.

The cellular localisation of nrg was determined and compared to that of its protein components nup98t (t for truncated) and smgGDS. cDNAs were cloned into the pEGFP-C2 mammalian expression vector to create green fluorescent protein (gfp) tagged proteins. The location of the gfp tagged proteins within transfected NIH-3T3 mouse fibroblast cells was visualised using confocal microscopy. Gfp-nup98t was located in a punctate pattern around the nuclear envelope. Gfp-smgGDS was found throughout the cytoplasm and was absent from the nucleus. The hybrid protein gfp-nrg was present throughout the cytoplasm but was also visible within specific subnuclear domains. Therefore the formation of nrg results in nuclear localisation of the normally cytoplasmic smgGDS protein. Nup98t has been shown by others to have strong transcriptional transactivation ability while smgGDS has been shown to be important in blocking apoptosis in thymocytes. It is therefore possible that nrg promotes leukemogenesis through two independent pathways. In the nucleus nrg may act to deregulate transcription pathways critical to normal T-cell development, while in the cytoplasm, nrg may act to increase T-cell survival by promoting an anti-apoptotic phenotype.